EFFECT OF SORAFENIB ON HUMAN COLON CANCER CELLS

Marwa Al Hassan
PhD Student, Faculty of Science, Beirut Arab University, Debbieh, Lebanon, MHassan@bau.edu.lb

Farah Reda
PhD Student, Faculty of Science, Beirut Arab University, Debbieh, Lebanon, farahreda87@hotmail.com

Ali Baltaji
Student, Faculty of Medicine, Beirut Arab University, Beirut, Lebanon, aliibrahimbaltaji@gmail.com

Julnar Usta
Professor, Faculty of Medicine, American University of Beirut, Beirut, Lebanon, justa@aub.edu.lb

Jamilah Borjac
Professor, Faculty of Science, Beirut Arab University, Debbieh, Lebanon, j.borjac@bau.edu.lb

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Abstract

ABSTRACT: *Sorafenib, a kinase inhibitor, is among the approved drugs for the treatment of radioactive iodine resistant thyroid carcinoma, primary kidney and liver cancers. Reported targets of Sorafenib include VEGFR, Raf family, and PDGFR belonging to the general class of tyrosine kinases. Blocking growth signals in kidney and breast cancers underlie one of the mechanisms of Sorafenib antitumor effects' leading to cell death. We hereby examine the effect of Sorafenib on human colon carcinoma cell-line HCT116. We also investigate the possible role of p53 in mediating this effect using mutant HCT116 p53-/- cells. Cultured wild and mutant cells are treated with Sorafenib (0-75μM) for 24 hr. This is followed by assessing the viability of cells using MTT and trypan blue exclusion assays. We also examined if Sorafenib mode of action is mediated by ROS. Levels of ROS were determined in the presence and absence of antioxidants using the colorimetric NBT assay. Our preliminary results show a concentration dependent decrease in viability (trypan blue) with an estimated EC50 of 10 and 25 μM for HCT116 and HCT116 p53-/- respectively. Compared to trypan blue, MTT results were similar in case of HCT116 p53-/- but were significantly different with HCT116. Furthermore we obtained a significant increase in level of ROS of: 37.11% and 31.30% for HCT116 and HCT116p53-/- respectively. However, 2 hours pre-incubation of cells with antioxidants, Trolox, N-acetylcysteine (NAC), and catalase, prior to Sorafenib treatment, exerted no different effect. No restoration of viability or decrease in generated ROS level was noted except when pre-incubated with NAC. Our preliminary findings show that Sorafenib action is independent of ROS level and p53 expression and further investigations on the mechanism(s) of Sorafenib action are ongoing.*

Keywords

Sorafenib, Colon carcinoma, Apoptosis, ROS, Cell motility

1. INTRODUCTION

Colorectal cancer, commonly known as bowel cancer, occurs in the colon, appendix, or rectum. CRC is the third most common cancer and known as the second most common cause of cancer death (Siegel, et al., 2020). From 2007 to 2016, incidence rates of colorectal cancer declined by 3.6% annually among adults 55 years of age and older, but increased by 2% annually among adults younger than age 55 (American Cancer Society, 2020).

Diverse risk factors for colorectal cancer (CRC) include genetic predisposition and incidence of inflammatory bowel diseases (Andersen & Jess, 2013). Moreover, studies have associated coronary heart disease with an increased risk of CRC (Wang, et. al., 2019). In addition, many bacterial and viral agents were proposed among the risk factors for CRC (Macrae, 2020).

Consistently, several distinct treatments and predictive measures are used to cure CRC (Iasonos, Chapman & Satagopan, 2016). Similar to different types of cancer, the treatment of colon cancer depends on its advancement stage. While surgery is generally curative at early stages; however when the cancer metastasizes, chemotherapeutic drugs are introduced in the treatment at later stages (Stein, Atanackovic, & Bokemeyer, 2011).

Over the last five decades, many chemotherapeutic drugs were used, of which is 5-fluorouracil (5-Fu), one of the most effective and most commonly used drugs to treat colorectal cancer. However, intrinsic and acquired resistance remains a main setback to its clinical efficacy (He *et.al., 2017).*

Combined chemotherapeutic regimens are currently being adopted for the treatment of colorectal cancer such as oxaliplatin with 5-FU or leucovorin. This drug combination; FOLFOX, is currently considered as the first-line chemotherapy strategy for metastatic CRC (Jeught, et al., 2018).

In the recent years, novel chemotherapeutic and immunotherapeutic drugs have been developed and used in the treatment of colorectal cancer. Among the monoclonal antibodies used are panitumumab that targets epidermal growth factor receptors (EGFR) blocking cell division and growth (Ruthann, et al., 2007), and bevacizumab that inhibits the activity of VEGF and thus inhibiting thus angiogenesis. These drugs have been used in combination with 5-FU (Pavlidis, 2013).

Sorafenib (Figure 1 showing structure), a drug approved for the treatment of patients with advanced renal cell (RCC) and hepatocellular carcinomas (HCC), has been demonstrated as the first molecular targeted agent with significant therapeutic benefits in advanced hepatocellular carcinoma (Wilhelm, et al., 2008; Bahman, et al., 2018). Sorafenib has a unique property compared to other anticancer drugs is its ability to induce ferroptosis in different cancer cell lines including kidney cancer cells (Caki-1, ACHN), colon cancer cells (HCT116, HT-29), and hepatocellular carcinoma cells (Lachaier, et., al., 2014).
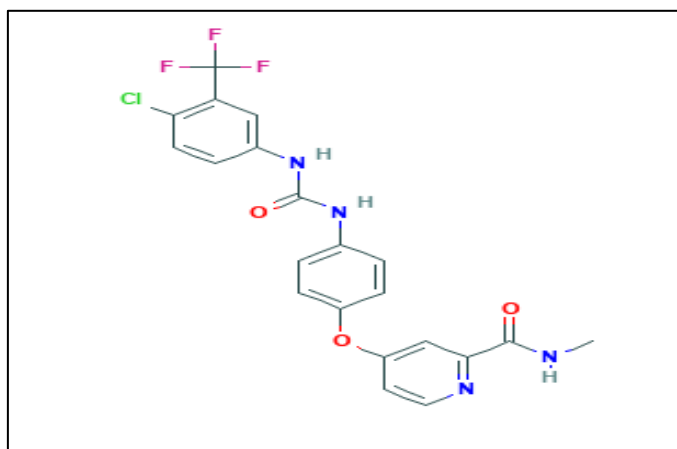

Fig.1: Chemical Structure of Sorafenib. ($C_{21}H_{16}ClF_3N_4O_3$, 4-[4-[[4-chloro-3 (trifluoromethyl)phenyl]-carbamoylamino]phenoxy]-*N*-methylpyridine-2-carboxamide) (PubChem, 2006).

Further studies have shown that combined administration of Celecoxib; a cyclooxygenase-2 inhibitor, and Sorafenib decreases production of phospho-AKT and increases apoptosis in HCC, thus, inhibiting tumor proliferation (Morisaki, et al., 2013).

Moreover, Sorafenib treatment of androgen-independent prostate cancer patients (AIPC) and prostate cancer cell lines (PC3, DU145 and 22Rv1) showed significant clinical effects and reduced cell viability by inducing apoptosis respectively (Ullen, et al., 2010). Sorafenib has demonstrated clinical effects in three independent phase II trials in patients with hormone refractory and metastatic prostate cancer (Ullen, et al., 2010).

Furthermore, Sorafenib was shown to inhibit the growth of the colon cancer cell lines SW480, HT29 and SW620, at a dose as low as 5µg/ml (Wehler, et.al, 2012). The combination of 5-Fluorouracil (0.5 mg/ml of 5-FU) and Sorafenib (5µg/ml) revealed additive effects in SW480 and HT29 cells (Wehler, et.al, 2012).

Knowing that the cell lines SW480 and SW620 exhibit mutated p53 gene (Lamy, et.al, 2010), it was stated that despite these mutations, depending on the cellular stress, the p53 protein may retain its ability to induce DNA repair mechanisms, apoptosis, and cell cycle arrest (Rochette, et.al, 2005). In this study, the effect of Sorafenib on two well-characterized in vitro human colorectal cancer cell lines with different p53 status is being investigated. P53, a tumor suppressor gene, is activated in response to stress and can promote the permanent removal of nascent cancer cells through the induction of various forms of cell death or senescence, responses that contribute to the ability of p53 to limit tumor development (Blagih, Buck & Vousden, 2020). In addition, cell-cycle arrest and apoptosis are the most prominent biological outcomes of p53 activation in cell culture experiments (Chen, 2016). Many studies showed that the p53 apoptotic function is important for preventing tumor development (Blagih, Buck & Vousden, 2020). Therefore, to study whether Sorafenib exerted an inhibitory effect in a p53-independent way, we chose the wild type cell line HCT116 and the mutant type HCT116 p53-/- as the objects of this study.

2. METHODOLOGY

2.1 Materials

Sorafenib was purchased from Santa Cruz Biotechnology, Dallas, TX, USA. Human colon cancer cells (HCT116, HCT116P53$^{-/-}$, SW948 and SW837) were obtained from American Type Culture Collection (ATCC), Manassas, VA, USA. Roswell Park Memorial Institute media ((RPMI, cat # R7388), Heat inactivated fetal bovine serum (FBS, cat # F9665), Phosphate Buffered Saline (10X PBS, cat # D1408), Penicillin-Streptomycin (PEN-Strep mixture, cat # L0022-100), Trypsin-EDTA 10X solution (cat # T3924), antioxidants N-acetylcysteine (NAC) (cat # A9165), Catalase (cat # 3515) and Propidium iodide (cat # P4170), and 6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich, USA.

The Cell Proliferation Kit I for viability assay (MTT, cat # 11 465 007 001) was obtained from Roche (Mannheim-Germany). Annexin V-FITC Apoptosis Detection Kit (cat # ab14085) was bought from Abcam and p -Nitro Blue Tetrazolium Chloride (NBT, cat # 6876) was from MP chemicals (CA-USA).

2.2 Preparation of Sorafenib

Sorafenib was dissolved in dimethyl sulfoxide (DMSO), at varying concentrations ranging between 5µM-100µM then aliquoted and stored at -20^0 C.

2.3 Culture conditions of Colon Cancer Cell Lines

HCT116, HCT116P53-/-, SW948 and SW837 colon cancer cells (CCC) were cultured in media supplemented with 10% fetal calf serum, 0.5% penicillin and streptomycin and incubated in humidified 5% CO2 incubator at 37°C. RPMI was the media used for HCT116 while DMEM was the media used for HCT116P53-/-, SW948 and SW937cells.

2.4. Cell Viability

Cell viability was evaluated using MTT assay and Trypan Blue exclusion test.

2.4.1. MTT assay

Viable cells were stained by using 3-(4-5 dimethyl thiazol-2yl)-2,5 diphenyl tetrazolium bromide (MTT) following the supplier's instructions. CCC (1×10^4 cells/well) were seeded in 96 well plates, then cultured under standard conditions and treated with Sorafenib (5 to 75µM) for 24 h. Viability of cells was compared to control cells treated with vehicle (DMSO, 0.1%). MTT reagent (10µl) was then added, incubated for 4 h followed by the addition of solubilizing reagent (100µl) and incubation overnight. Cell viability was measured by examining the cellular reduction of the yellow MTT to the insoluble purple formazan form by the metabolically active cells. Absorbance of the developed color was measured using the ELISA reader Multiskan EX (Thermo) at 595 nm. Percent viability of treated cells was calculated as follows:

% Viability = [Abs (treated cell)/ Abs (control)] ×100 (Katerji, et. al, 2017)

2.4.2. Trypan blue exclusion assay

The effect of Sorafenib on the viability of the CCCs was also determined by trypan blue exclusion test. Cells (1×105/ml/well) were seeded in 12 well plates, treated with Sorafenib (5µM-75µM) for 24 h. They were then trypsinized, collected by centrifugation, resuspended in media: trypan blue solution (1ml:10µl) and counted using hemocytometer under a light microscope.

% Viability = [number of treated unstained cells/ total number of stained and unstained cells] ×100 (Ballout, et. al, 2020)

2.5. Nitroblue Tetrazolium (NBT) Reduction Assay

CCCs were seeded in 96 well plates at a density of 1×10^4/100µl for HCT116 and 1.2×10^4 for HCT116P53$^{-/-}$. Cells were then treated with Sorafenib for 24 h, each at its corresponding EC50. The level of reactive oxygen species (ROS) was indirectly determined using the nitro-blue tetrazolium (NBT) assay (Muñoz et al., 2000). In short, freshly prepared NBT (1 mg/ml, 100µl) was added to control and Sorafenib treated cells, incubated for 1hr, then washed with methanol (100µl/well), then left to dry at room temperature. The formed formazan crystals were solubilized by consecutive addition of KOH (2M, 120µl) and DMSO (140 µl). Absorbance of the developed blue turquoise color was read at 630 nm using Elisa Multiskan EX Reader.

The % of reduced NBT and % of generated ROS were determined as follows:

%NBT reduction = (Abs of Sorafenib-treated cells /Abs of control) ×100
% ROS production = 100 - %NBT reduction

The level of ROS generated is inversely proportional to the level of NBT reduced (Al Wafai, et. al, 2017).

2.6. Effect of Antioxidants on Sorafenib Treated Colon Cells

The possible protective effect of the antioxidants NAC, Trolox and enzyme catalase on viability and ROS production in Sorafenib treated colon cancer cells was assessed. Seeding was done as described before in the respective assays. CCC were pretreated for 2hrs with NAC (5mM), Trolox (100µM), or Catalase (8 Units) followed by Sorafenib treatment for 24 hrs. Restoration of viability and the ability to reduce NBT were then determined.

2.7. Wound Healing Assay

Wound healing assay was performed to qualitatively determine the effect of Sorafenib on cell migration. CCCs were seeded in 6 well plates at density of 20x104 cells /well and a scratch wound was made in the monolayer with a sterile pipette tip. Cells were

then washed with PBS to remove debris and new media were added. Phase-contrast images of the wounded area were taken at 0 and 24 hours. Wound widths were measured at 3 different points for each wound, and the average rate of wound closure was calculated in µm/hr (Karakuş, et al., 2018).

2.8. Apoptosis Analysis

Annexin-V is a protein that binds the surface phospholipids (PS) in apoptotic cells. Annexin-V/propidium iodide double staining was used to measure the percentage of apoptotic cells according to the annexinV FITC apoptosis detection kit (ABCAM). HCT116 and HCT116-P53-/- cells were cultured in 6-well plates at a density of 1×10^5 cells/ml, and treated for 24hr with Sorafenib at (EC50). Cells were then trypsinized, washed with PBS, re-suspended in Annexin-V-FITC and PI containing binding buffer (20 µl of 1mg/ml), and incubated for 5 min at room temperature in the dark. The Annexin-V FITC−/PI− cell population was considered normal, whereas the Annexin-V FITC+/PI− and Annexin-V FITC+/PI+ were indicative of early and late apoptotic cells respectively. Five thousands ungated events were collected for each sample. Flow cytometry data were plotted and analyzed by the fluorescence Guava Easy Ctye8 Flow Cytometer (Millipore/Merck, CA, USA).

2.9. Effect of Sorafenib on SW948 and SW837 Cell Lines

To study the effect of Sorafenib on Colon cancer cells with two different P53 mutations in Human colorectal adenocarcinoma cell lines, SW948 (G117fs) & SW837 (R248W) were used. Cells were cultured with DMEM media supplemented with10%FBS and 1%PS, seeded in 96-wellplates (1×10^4 cells/well) and 12-well plates (1×10^5 cells/well) and treated for 24h with the appropriate concentrations of Sorafenib. Viability and NBT assay were examined in Sorafenib treated SW948 and SW837 cell lines.

2.10. Statistical Analysis

Microsoft Excel independent student t-test was used to determine the statistical significance. P-values ≤ 0.05 were considered significant. For each parameter tested, at least three experiments were performed with triplicate determinations in each. Moreover, for each parameter, statistical significance relative to the control and inter-categorical significance were analyzed using the t-test.

3. RESULTS

3.1. Sorafenib Decreases Viability of Human Colon Cancer Cells

The effect of Sorafenib on the viability of HCT116 and HCT116P53-/- was assessed using both MTT and trypan blue exclusion assays (Figures 2A and 2B). A dose dependent decrease in cell proliferation was obtained in both assays.

Using MTT assay, a 50% cell death (EC50) occurred at 50µM and at 35µM for HCT116 and for HCT116P53-/- respectively (Figure 2A). Trypan blue exclusion assay showed that Sorafenib exerted the same effect at lower concentration. The obtained EC50 were 10µM and 25µM for HCT116 and for HCT116P53-/- respectively (Figure 2B), which shows that the wild type HCT116 is more sensitive to Sorafenib.
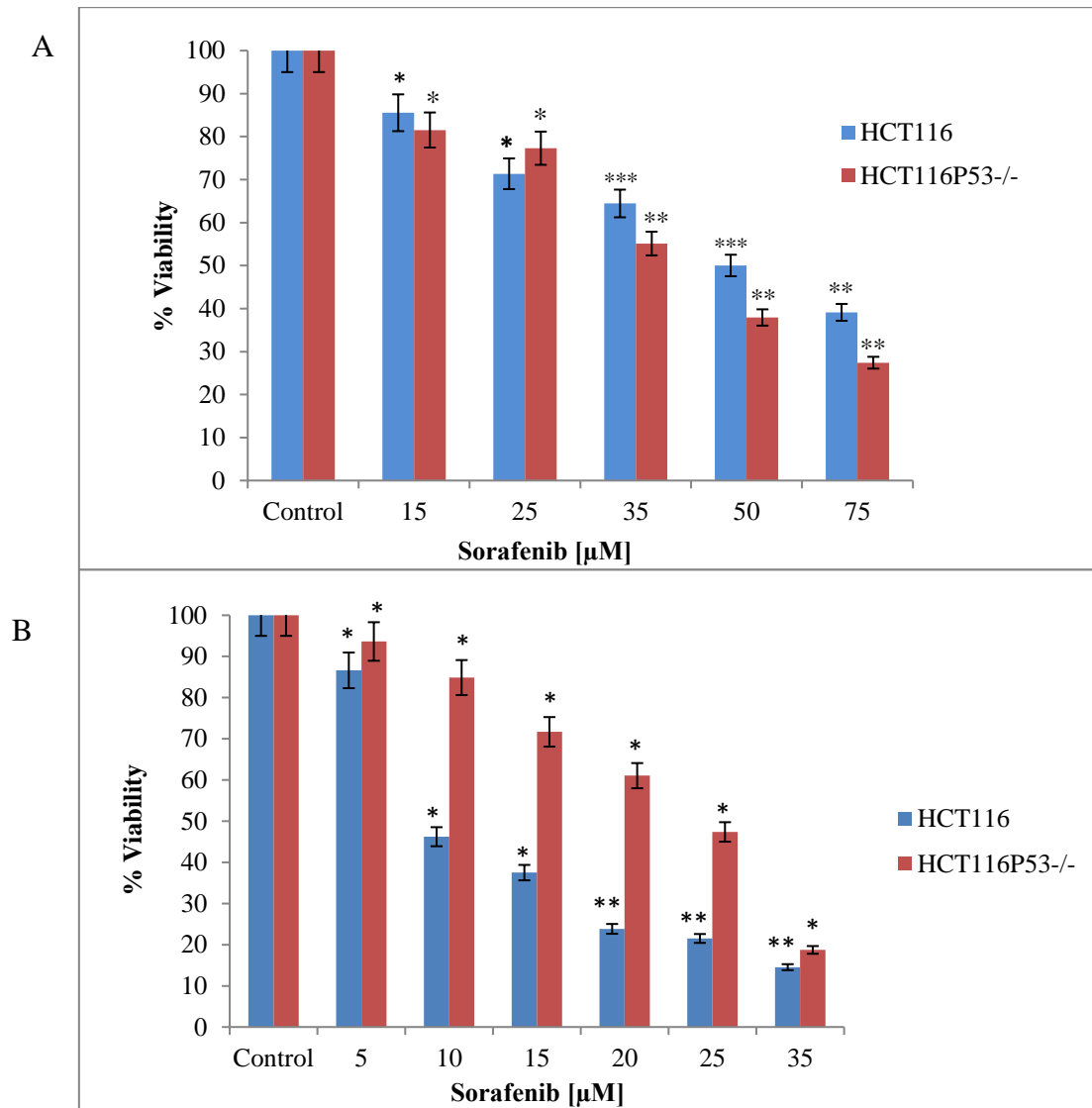
Fig.2: A) Effect of varying Sorafenib concentrations on viability of HCT116 and HCT116p53$^{-/-}$ cells using MTT assay. B) Effect of varying Sorafenib concentrations on viability of HCT116 and HCT116p53$^{-/-}$ cells using Trypan Blue Exclusion Assay. Data presented are the mean ± SEM of 9 determinations from three different experiments. Asterisks on bars represent significance relative to the control. (*), (**), and (***) correspond to $P<0.05$, 0.01, and 0.001, respectively.

3.2. Sorafenib Induces ROS Production

Treating HCT116 and HCT116p53-/- with Sorafenib (5 to 50 µM) significantly (p<0.05) increased ROS production in a dose dependent manner. A 25.6% to 53% increase in ROS production was observed in HCT116 and 9% to 52% in HCT116p53-/- after 24 hours of treatment (Figure 3).
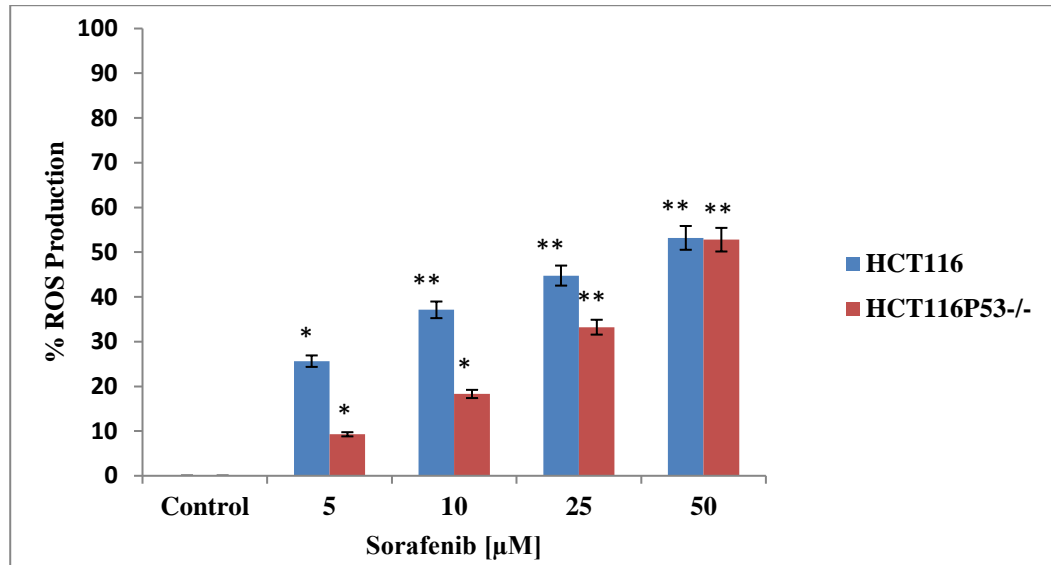
Fig.3: Effect of Sorafenib on ROS Production. Cells were treated with Sorafenib at different concentrations for 24-h. Data presented are the mean ± SEM of 9 determinations from three different experiments. Asterisks on bars represent statistical significance relative to the control. (*) and (**) correspond to $P<0.05$ and 0.01 respectively.

3.3. Effect Of Antioxidants On Viability Of Sorafenib Treated Colon Cancer Cells

We next examined the possible protective effect of the antioxidants (NAC, Trolox), and the catalase enzyme in restoring viability of Sorafenib-treated CCC using MTT assay. Pre-treatment for 2hrs with each of the antioxidants Trolox, NAC and the catalase enzyme exhibited no significant protective effect as no restoration in viability was observed in both cell lines as shown in Figure 4.
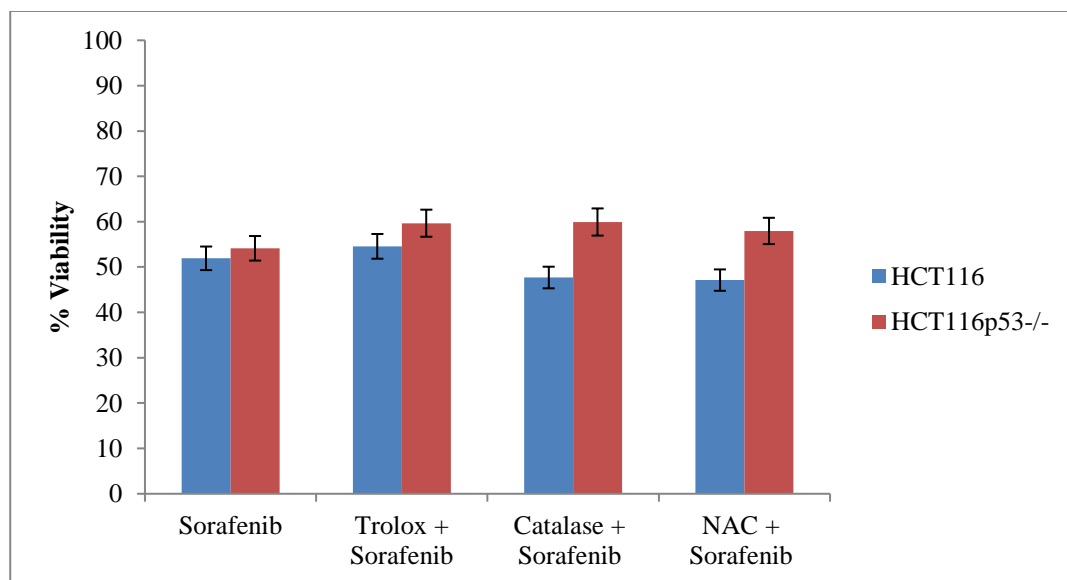

Fig.4: Effect of Antioxidants (Trolox, NAC, and Catalase) on viability of Sorafenib treated HCT116 and HCT116p53-/- cells using MTT Assay. Colon Cancer cells were pre-incubated for 2h with NAC (5mM), Catalase (8U) and Trolox (100µM) prior to Sorafenib treatment at EC50 and incubation for additional 24-h. Data presented are the mean ± SEM of 9 determinations from three different experiments.

3.4. Effect of Antioxidants On ROS Production of Sorafenib Treated Colon Cancer Cells

We next examined the effect of the antioxidants (NAC, Trolox) and the catalase enzyme on prevention of ROS generation in Sorafenib-treated CCC using NBT assay. Pre-treatment for 2hrs with each of the antioxidants Trolox, NAC and Catalase exhibited no protective effect on either cell lines (Figure 5).
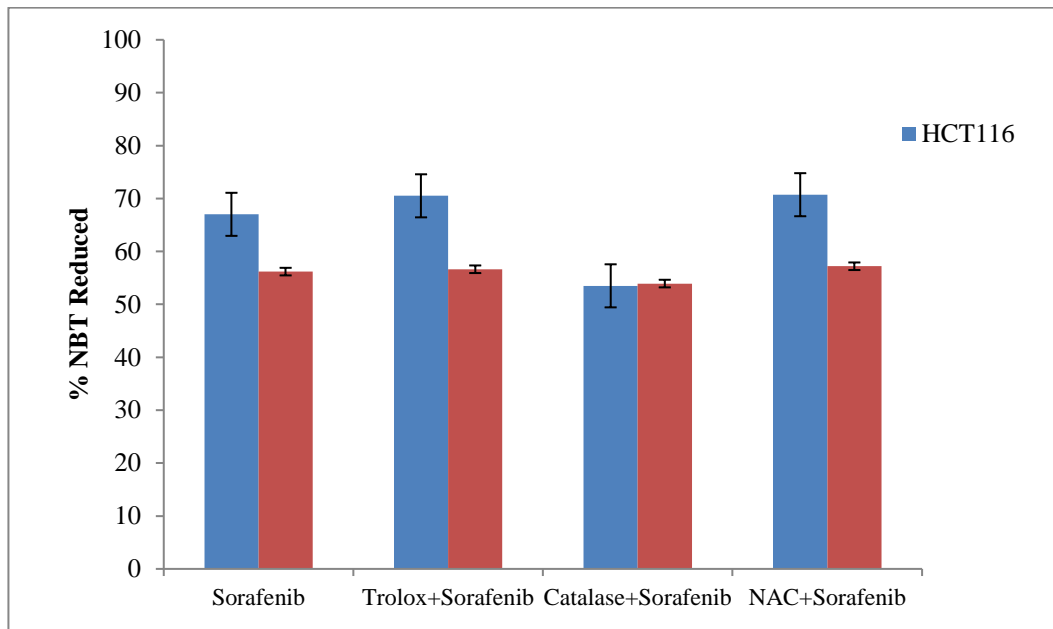

Fig.5: Effect of Antioxidants (Trolox, NAC) and Catalase on NBT reduction of HCT116 and HCT116p53$^{-/-}$ using NBT Assay. Colon Cancer cells were pre-incubated for 2h with NAC (5mM), Catalase (8U) and Trolox (100μM), prior to Sorafenib treatment at EC50 for 24-h. Data presented are the mean ± SEM of 9 determinations from three different experiments.

3.5. Sorafenib Induces Apoptosis of Colon Cancer Cells

To investigate whether cell death observed following Sorafenib treatment in the two colon cancer cell lines occurs via apoptotic or necrotic mechanisms, cells were stained with fluorescin Isothiocyanate (FITC)-conjugated Annexin-V antibody (Annexin V-FITC) and PI followed by flow cytometry.

Following Sorafenib treatment (24hrs) an increase in the percentage of AnnexinV and PI stained CCC cells was obtained, compared to control, indicating late apoptosis. As shown in Figure 6, the percentage of cells in late apoptosis were 40% and 60% for HCT116 and HCT116p53-/- respectively, showing 8 fold increase in the wild type HCT116 when comparing treated to control, and 4 fold increase in the mutant type HCT116p53-/-. Therefore, upon Sorafenib treatment, HCT116 are more sensitive to cell death than HCT116p53-/- .
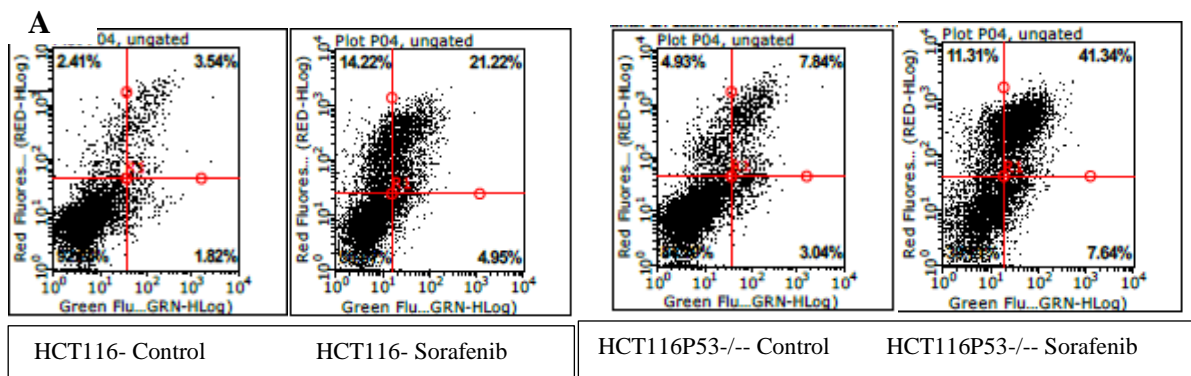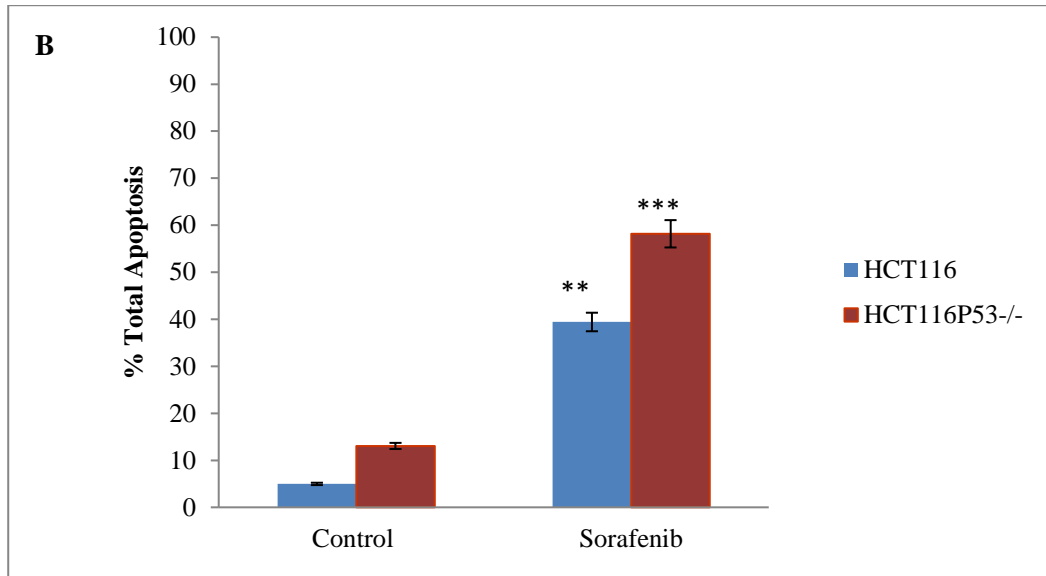
Fig. 6: Apoptosis assessment of Sorafenib-treated colon cells. Figure 6A shows representative Annexin-V FITC:PI histograms of Sorafenib-treated cells compared to control for HCT116 and HCT116p53$^{-/-}$ cells. Alive cells are Annexin V− /PI−, dead cells are Annexin V− /PI+, early apoptotic cells are Annexin V+ /PI− and late apoptotic/necrotic cells (Annexin V+ /PI+). Figure 6B shows the data presented as the mean ± SEM of 3 different experiments. Asterisks on bars represent significance relative to the control (**), and (***) correspond to $P < 0.01$, and 0.001, respectively.

3.6. Sorafenib Affects Cellular Motility

The effect of Sorafenib on the motility of the colon cancer cells was investigated in a 2D system using the wound healing assay. Our results show that Sorafenib induced a decrease in motility, causing a 2.5 fold decrease in wound closure in HCT116p53$^{-/-}$ cells compared to untreated cells (Figure 7). This experiment was also performed on the wild type HCT116, which did not adhere to the culture plate after treatment with Sorafenib.
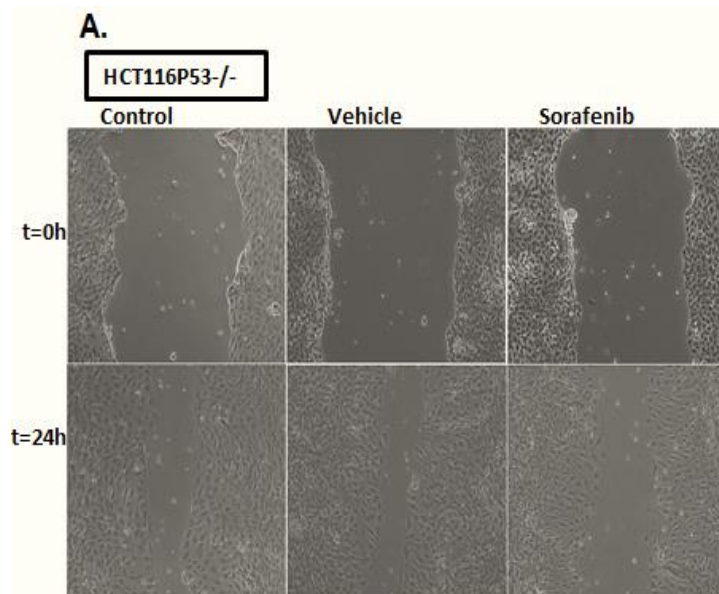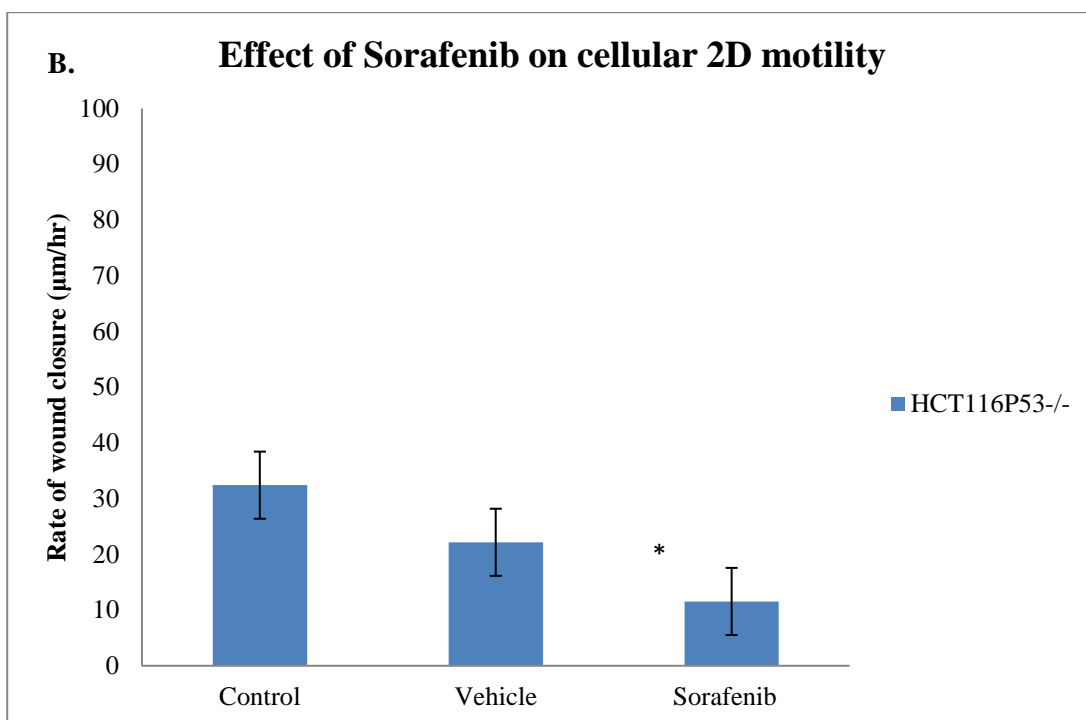
Fig.7: Sorafenib inhibits cellular motility. Monolayers of untreated and Sorafenib-treated HCT116p53$^{-/-}$ cells grown in plates were wounded. Pictures of cells were taken at 0 hr (upper micrographs) and 24 hrs (Lower micrographs) post wounding at the same frame. Width of single wound was measured at 3 distinct points, and average rate of wound closure was calculated in μm/h. Data are the mean -/+ SEM from 3 different wound healing experiments.

3.7. Effect of Sorafenib On SW948 And SW837 Cells

Similar viability profile to that of HCT116 and HCT116p53-/- was obtained in the p53 wild type SW948 and the mutated SW 837 cells treated with Sorafenib. Using MTT assay, a 50% cell death (EC50) occurred at 35μM and 25μM for SW948 and SW837 cells, respectively (Figure 8A). Trypan blue exclusion assay showed that Sorafenib exerted the same effect at lower concentration. The obtained EC50 was 10μM for both cell lines (Figure 8B). Sorafenib treatment significantly decreased cell ability to reduce NBT in a dose dependent manner in SW948 and SW837 cells, 74.6% and 84.0% ($p < 0.05$), respectively at EC50 (Figure 8C). These findings indicate a significant increase in ROS level, 25.4% and 16% ($p < 0.05$) in SW948 and SW837, respectively at EC50 as shown in Figure 8C.
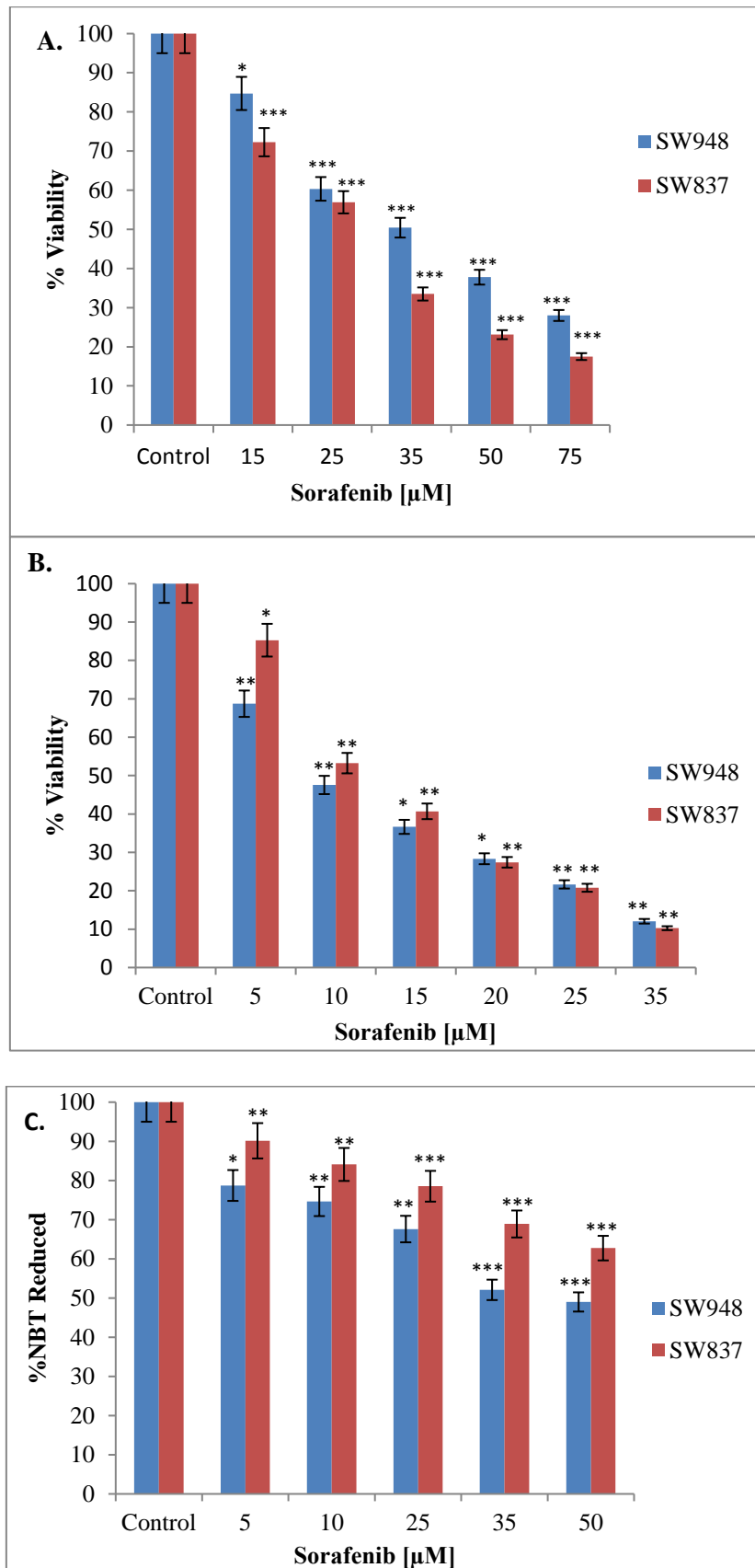
Fig.8: Effect of Sorafenib treated SW948 and SW837 cells on:(A) viability using MTT assay; (B) Trypan blue exclusion test, (C) ROS level using NBT reduction assay The data presented as the mean ± SEM of 3 different experiments. Asterisks on bars represent significance relative to the control (*), (**), and (***) correspond to $P < 0.05$, 0.01, and 0.001, respectively.

4. DISCUSSION

Sorafenib, a novel drug used for the treatment of different types of cancer, has so far demonstrated its antitumor activity in patients with hepatocellular carcinoma, sarcoma and renal cell cancer (RCC) (Strumberg, 2005). This study was undertaken to investigate the *in vitro* effect of Sorafenib on the colon cancer cell lines HCT116 and HCT116P53$^{-/-}$.

Our study reports that Sorafenib treatment showed a concentration dependent decrease in viability (trypan blue) with an estimated EC50 of 10 µM for HCT116 and 25 µM for HCT116 p53$^{-/-}$ which was comparable with other studies reporting toxicity of Sorafenib at 10µM (Inoue et. al, 2011). Compared to trypan blue, MTT results were similar in case of HCT116 p53$^{-/-}$ but were significantly different with HCT116. Taking into consideration that trypan blue is a qualitative assay determining the number of viable cells based on the cell membrane integrity (Strober, 2001), the MTT assay measures mitochondrial function by monitoring the effect of Sorafenib, on the activity of mitochondrial dehydrogenase. MTT assay measure cellular viability through the mitochondrial activity (van Meerloo et al., 2011). Thus, this discordance may be attributed to the possible interference of Sorafenib with the MTT assay.

Sorafenib was cytotoxic to the two cell lines used and inhibited their growth at 10-25 µM (EC50 from Trypan blue exclusion test), which shows that the two cell lines HCT116 and HCT116P53$^{-/-}$ were responsive to Sorafenib, but the wild type HCT116 was more sensitive to the drug. These concentrations were similar to those obtained on CHLA-90 neuroblastoma cancer cells (10µM) obtained by Reynolds et al. (2013) where Sorafenib also showed anti-proliferative activity. Another study done by Cervello et al. (2012) showed a substantial dose-dependent decrease in cell viability in the two tested cell lines HepG2 and Huh7, where after 24hr of treatment with Sorafenib, the IC50 values were 12 µM in HepG2 cells and 11.3µM in Huh7 cells (Cervello et al, 2012).

Previous studies have shown that reactive oxygen species (ROS) play an important role in cancer (Prasad, et. al, 2017). Knowing that low levels of ROS could be beneficial, its excessive accumulation can induce cancer. One of the main characteristic of cancer cells that differentiate them from other normal cells is their ability to produce high levels of ROS and their increased dependence on an antioxidant defense system (Prasad, et. al, 2017). Our data shows that Sorafenib significantly decreased NBT reduction in the two cell lines HCT116 and HCT116p53-/- treated cells indicating an increase in ROS level. Furthermore, our results did not show a restoration of viability when pre-treated with antioxidants, and there was no significant change in ROS generation upon the pre-treatment of the two cell lines HCT116 and HCT116P53$^{-/-}$ with NAC and Trolox. However, Wan et al. showed that ROS generation was markedly abrogated when colon cancer cells HCT116 were pretreated with NAC, then treated with a combination Sorafenib and tetrandrine (Wan, *et al., 2013)*. Glutathione system is considered the cells' first line defense against extracellular ROS. In our study NAC that is not only considered as a ROS scavenger, but also one of precursors of glutathione synthesis (Li, et. al, 2016) was not able to rescue the HCT116 cells implying that cell death is not mediated through ROS production and rather ROS production is a consequence leading to cell death.

An increase in ROS production leads to genotoxic damage, which may lead to apoptosis or necrosis. Apoptosis is programmed cell death type I. Apoptosis plays an important role in maintaining cellular function in various tissues and organs (Tower., 2015). In our experiment, we evaluated apoptosis using Annexin V and PI double staining followed by flow cytometry. Our results indicate that Sorafenib significantly induces apoptosis in HCT116 and HCT116P53$^{-/-}$ cells showing 8 fold increase in the wild type HCT116 when comparing treated to control, and 4 fold increase in the mutant type HCT116p53$^{-/-}$, indicating that both cell lines have the same response to Sorafenib, with higher sensitivity seen in HCT116. Compared to other studies (Zhoa et al., 2013), Sorafenib induced apoptosis in HCC cell lines, specifically Huh7 and LH86 cells after 24hrs. Therefore, in this study, we hypothesized and presented partial evidence that p53 status in colon cancer cells may predict their sensitivity toward Sorafenib. P53, the tumor-suppressor protein, is a master regulator of apoptosis, in response to cellular stress (Farnebo et al., 2010).

Previous studies showed the role of p53 in the differential sensitivity to a combined treatment of Sorafenib and OSU-2S on HepG2 cells and Hep3B cells, by abrogating the sensitivity of HepG2 cell by p53 knockdown and rendering Hep3B cells much more sensitive by p53 overexpression (Omar et. al, 2016). To test whether the effect of Sorafenib is specific to HCT116 and HCT116p53 $^{-/-}$ cells only or not, we determined its effect on two additional cell lines,

the SW948 and SW837cells. In our study, we showed that HCT116 was more sensitive to Sorafenib than HCT116p53$^{-/-}$ with an EC50 of 10 µM and 25µM, respectively. Sorafenib treated SW948 and SW837 cells had similar effects to those of HCT116 and HCT116p53 $^{-/-}$cells in terms of viability (EC50 = 10µM), and ROS generation implying the involvement of p53 in the sensitivity toward Sorafenib.

Our findings further revealed that Sorafenib inhibited the 2D cell motility of HCT116p53 $^{-/-}$ colon cancer cells. This property has been previously reported in other cancer models including ovarian cancer (Park, et. al, 2017) and hepatocellular carcinoma (HCC) (Yoshida, et. al, 2017). For instance, Sorafenib was shown to inhibit the migratory activity of EGF-treated SK-OV-3 and Caov-3 cells (Park, et. al, 2017). Sorafenib was also shown to suppress the cell motility of HLF cells (Yoshida, et. al, 2017). This is thus the first evidence of Sorafenib's antimigratory effects in CCCs in 2D, in vitro. In addition, while performing wound healing experiment, HCT116 did not adhere to culture plates after Sorafenib treatment, and this was comparable to a previous study that supports our findings, where HCT116 cells did not adhere to culture plates pre-coated with fibronectin after lactose and water soluble papaya pectin (3PP) treatments (do Prado, et. al, 2017). Hence, the differences in cell migration in both cell lines, wild type and mutant type could be related to the microenvironment that likely plays a crucial role in tumor growth and metastasis.

Taken together, these findings confirm that Sorafenib induces apoptosis and inhibit proliferation in ROS and P53 independent manner.

5. CONCLUSION

In summary, Sorafenib, independently from p53, exerted an anti-cancer effect on colon cancer by an increase in ROS production and inducing apoptosis. Furthermore, our results add to the growing evidence supporting the role that Sorafenib plays as anti-tumor agent.

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